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## Conducting pyrolysed carbon scaffold for tissue engineering

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This work presents the fabrication, characterization and testing of pyrolysed three-dimensional (3D) porous carbon materials as potential novel 3D conducting scaffolds (3D-CS) for tissue engineering applications.

Due to the multifunctional nature, carbon nanomaterials are becoming increasingly attractive, offering numerous opportunities to design novel sensors, drug delivery systems and scaffolds for tissue engineering<sup>1</sup>. For tissue engineering, carbon nanotubes (CNT) have been used to mechanically stabilize commonly used “soft” scaffold materials such as hydrogels and fibrous scaffolds<sup>2</sup>. Moreover, electrically conductive hydrogel based scaffolds have been demonstrated using CNT composites of these otherwise nonconductive polymers, enabling electrical stimulation of e.g. neural stem cells, resulting in improved action potentials and differentiation into functional neural networks<sup>3</sup>. Very recently, graphene foam (GF) - a 3D porous carbon structure - has been suggested as a new promising conductive scaffold that may incorporate, in the same structure, topographical, chemical and electrical cues<sup>4</sup>. We have recently shown that it is possible to precisely pattern SU-8 photoresist into high-aspect ratio sub-micron 3D pillar scaffold structures<sup>5</sup> that can be pyrolysed into their corresponding electrochemically active 3D carbon pillar scaffold structures<sup>6</sup>. In this study, we will demonstrate how scalable both randomly and structurally controlled porous conducting 3D carbon scaffolds can be generated through the pyrolysis of correspondingly porous polymer scaffolds of various polymers and copolymers. The idea is here demonstrated for a randomly structured PDMS sponge generated from a sacrificial carbohydrate template<sup>7</sup> (in this case a simple sugar cube). The biocompatibility of the 3D-CS was investigated and confirmed using live/dead cell imaging of cells grown on the scaffold for 3 days, and its potential suitability as an electrically active scaffold was demonstrated using electrochemical impedance spectroscopy (EIS).

The procedure for fabricating the 3D-CS from a sugar cube is shown in Fig. 1: (1) A randomly porous sugar cube was placed in a container, (2) pre-cured PDMS was poured into the container, which was (3) absorbed into the sugar cube by capillary forces in a vacuum desiccator for 1 hour. (4) The pre-cured PDMS was cured in an oven at 80 °C for 2 hour. (5) The sacrificial sugar in the sugar cube was then dissolved in water overnight, whereby (6) a randomly porous PDMS sponge was obtained. (7) The porous PDMS sponge was thereafter pyrolysed in a furnace at 900 °C for 60 min in N<sub>2</sub> atmosphere, resulting in a highly porous and hydrophilic 3D-CS, as shown in Fig. 2.

Without any further modification, the 3D-CS was sterilized and used for culturing of endothelial cells (HUVEC), which were allowed to perfuse through, then attach and grow for 3 days on and inside the 3D-CS. Fig. 3 depicts live/dead immunostaining (Dapi) after 3 days of cell culturing, showing cytoskeleton and the nucleus with no indication of dead cells, demonstrating that the support is biocompatible even without normally necessary modification with extra cellular matrix protein coating. The EIS spectra in Fig. 4, clearly indicates that the 3D-CS has inherent electrical conductivity, and thus its potential applicability for electrical stimulation of cells and/or as an electrical sensing scaffold.

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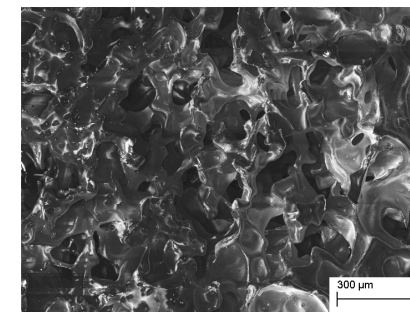
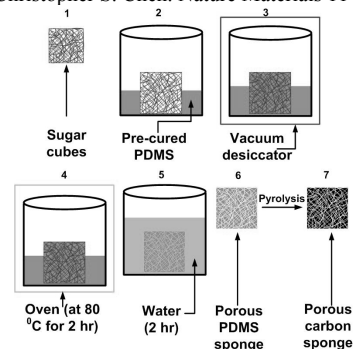


Figure 1. Fabrication of a conducting randomly porous carbon scaffold from a sugar cube.

Figure 2. SEM images of the fabricated porous carbon scaffold (3D-CS).



Figure 3. Live/dead cell imaging after 3 days of HUVEC cell culturing on 3D-CS in Fig. 2. Lighter zones-nucleus (blue in color), greyer zones-cytoplasm (green in color).

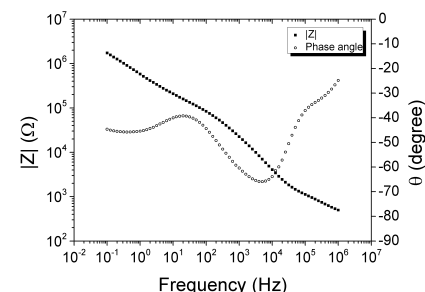


Figure 4. Electrochemical impedance spectrum (Bode plot) obtained with the 3D-CS seen in Fig. 2.

